DEFAULT VALUES AND READ-ACROSS PROPOSAL FOR DERMAL ABSORPTION BASED ON HUMAN SKIN IN VITRO STUDIES DATASET WITH PESTICIDES

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Abstract

The skin is the main route of exposure to plant protection products for operators, workers, residents, and bystanders. Assessing dermal absorption is key for evaluating pesticide exposure risks. However, conducting in vitro dermal absorption studies using human skin is resource-intensive. Therefore, establishing realistic regulatory default values is essential for non-dietary risk assessment and ensuring safety. This project analysed 468 in vitro dermal absorption studies with human skin compiled by ProHuma. Combined with a prior database by Aggarwal et al. (2015), the dataset comprised 759 GLP-compliant studies covering 25 formulation types and 248 active substances at various concentrations. The assessment confirmed earlier findings that dermal absorption shows no correlation with molecular weight or log Pow and does not increase linearly with dilution. Based on the comprehensive ProHuma/ECPA dataset, dermal absorption default values are proposed: 2% for solid concentrates, 5% for liquid concentrates, 30% for solid dilutions, and 34% for liquid dilutions. These values, derived conservatively using the 95th percentile, better represent formulations and agricultural use cases, reducing the need for unnecessary studies. The project also suggests read-across between different formulation types. By proposing more realistic default values and read-across approaches, it streamlines decision-making and supports safety in agriculture.

KEY WORDS

Dermal absorption, *in vitro* human skin, risk assessment, default values, plant protection products, agrochemicals, pesticides, OECD 428

1. Introduction

In this project, a homogeneous combined dataset with 759 *in vitro* dermal studies using human skin (ProHuma/ECPA dataset) was obtained by evaluating 488 studies conducted mainly from 2014 to 2019 (ProHuma dataset) in combination with the dataset from the European Crop Protection Association - ECPA, currently CropLife Europe (Aggarwal *et al.,* 2015), which is the result of a combined dataset of studies described in two papers published in 2014 and 2015. A total of 291 studies are listed in the Zenodo repository[1] and were considered reliable to be used in this project.

After exclusion of studies not meeting the quality criteria, a proper statistical approach was used to assess a total of 2008 observation values derived from the evaluated studies to compose the biggest dataset of *in vitro* human skin dermal absorption studies for pesticides currently known.

1.1. Background

The skin is a multi-layered organ comprising three main layers: epidermis, dermis, and hypodermis. Together they form a natural barrier of protection to the environment (temperature, dehydration, bacteria and viruses, injuries, etc.) and the absorption of exogenous substances, including chemicals and pesticides[2]. The upper layer is the epidermis, a non-vascular layer that has a general protective role by acting as a barrier to penetration in the lower layers where vascularisation occurs, which could lead to a potential systemic exposure to the agent that has penetrated through epidermis (WHO, 2006).

The primary cells – keratinocytes – are produced in the deepest layer of the epidermis, the stratum basale. They migrate within a few weeks up to the stratum corneum. In the process, keratinocytes lose their nucleus and become flattened but increasingly cornified cells forming a barrier to the environment and exogenous agents. Finally, a process of normal epidermal desquamation leads to the renewal of the cells and eliminates the residues trapped in the epidermis (WHO, 2006).

When an exogenous agent is in contact with the skin, the agent must penetrate the entire epidermis and the basement membrane to reach the capillaries in the dermis and the systemic circulation. Penetration may also occur via sweat and sebaceous glands and hair follicles directly to the dermis (WHO, 2006).

[1] https://zenodo.org/record/3378822#.YDjL69Wg_IG

[2] Throughout the text, the terms 'pesticide', 'agrochemical', and 'plant protection product' (PPP) will be used interchangeably.

Dermal absorption is one of the most relevant parameters to assess the risk consecutive to human exposure to pesticides as skin is usually the main route of human exposure during mixing, loading, application, and other post application activities (monitoring, reaching, picking, cutting, etc) (OECD, 2019).

Most plant protection products are applied by spraying a water dilution of different concentrations of commercial formulations (Aggarwal *et al.*, 2014). Exposure to diluted formulations is usually associated to a higher dermal absorption as penetration through the skin tends to increase with the water content. Thus, a higher internal dose and potential risk are expected when comparing exposure to dilutions and the neat formulation (EFSA, 2017).

In a typical OECD test-guideline compliant *in vitro* dermal absorption study using human skin (OECD 428), a pesticide (neat formulation/concentrate) and one or more spray dilutions containing – preferably – radiolabelled active substance are applied to the human skin for at least 6-10 hours to match a representative working day. Dermal absorption is determined through the material collected by continuous measurements for up to 24 hours after the initial skin contact (OECD, 2019).

In the European Union, when no experimental data are available on the formulation, the European Food Safety Authority – EFSA Guidance on Dermal Absorption (2017), defines the following default values to be used: for organic solvent-based[3] or other formulations[4], 25% for concentrates and 70% for dilutions; for water-based/dispersed[5] or solid formulations[6], 10% for concentrates and 50% for dilutions.

These values are based on a heterogeneous database which includes, e.g. studies with exposure durations not representative of a working day, variable recovery criteria and rat data. In combination with the limited and restricted read-across possibilities according to the EFSA guidance, it triggers the need for dermal absorption studies for almost all formulations – including virtually identical formulations of the same active substance – even when expert judgment indicates that reliable surrogate data are available (Aggarwal *et al.,* 2015).

[3] Formulation types from this grouping as per EFSA (2017): emulsifiable concentrate (EC), emulsion, oil in water (EW), suspo-emulsion (SE), dispersible concentrate (DC), oil miscible liquids (OL/OF), oil-based suspension concentrates (OD), emulsion for seed treatment (ES), microemulsion (ME).

[4] Bait concentrate (CB), capsule suspension (CS), gel for direct application (GEL/GD), bait, ready for use (RB), mixture of capsule suspension and suspension concentrate (ZC), seed coated with a pesticide (PS), experimental solution of active substances in solvent (AI).

[5] Soluble concentrate (SL), suspension concentrate (SC), flowable concentrate for seed treatment (FS), flowable (FL) (=SC).

[6] Wettable powder (WP), water-dispersible granules (WG/WDG), water-soluble granules (SG), water-soluble powder (SP), powder for dry seed treatment (DS).

ECPA proposes the following dermal absorption default values: 6% for liquid and 2% for solid concentrates, irrespective of the active substance concentration, and a single dermal absorption default value of 30% for all spray dilutions, irrespective of the formulation type, as well as possibilities to read-across formulations. The studies evaluated were compliant with the principles of Good Laboratory Practices – GLP and guideline OECD 428 in order to ensure having a robust, homogeneous and comprehensive database complying with the EU regulatory requirements.

In Brazil, according to the regulation "Resolução da Diretoria Colegiada 294" (RDC 294) from the Brazilian Health Regulatory Agency (ANVISA, 2019), studies to estimate *in vitro* or *in vivo* dermal absorption or both should be submitted. When no studies are available, the use of default values or read-across can be used upon scientific and technical basis. However, currently there is no clear process in Brazil for the use of human tissue and there are a limited GLP laboratories with expertise to run those studies with radiolabeled material.

The current publication presents the results of a work initiated by ProHuma, a consortium gathering several companies of the Brazilian pesticides sector. It endorses the previous conclusions by Aggarwal *et al*. (2015) by expanding the dataset with newly available dermal absorption studies performed up to 2019 ('ProHuma/ECPA dataset').

1.2. Objectives

To assess the impact of different parameters on dermal absorption by analyzing an additional dataset of 468 *in vitro* studies and a combined ProHuma/ECPA dataset with 759 *in vitro* studies and propose robust dermal absorption default values and read-across possibilities.

2. Methods

2.1. Data collection

Data collection from ProHuma dataset and data analysis from this project was performed by qualified professionals from third-part and independent consultancy companies.

Study reports granted by companies associated to ProHuma are considered confidential data. A Brazilian consultancy was responsible to extract the data for the ProHuma dataset and has established a confidentiality agreement with each associated company. ProHuma did not have any access to the reports nor any associate member. The data was compiled in a spreadsheet with the same fields from the dataset available from Zenodo.

The spreadsheet containing the information from ProHuma dataset was shared with a specialized Europe-based consultancy, who combined with the ECPA dataset. This consultancy was responsible for the data analysis and reporting with the support of an additional independent statistician expert.

ProHuma dataset includes studies covering 22 different formulation types and 190 active substances and the combined ProHuma/ECPA dataset, 25 different formulation types with 248 active substances (Table 1). Formulation types that were not classified as organic solvent-based, solid or water-based/dispersed, were grouped as “other types” as there was no specific characterization for these. The concentration range of the active substances in the combined dataset was 0.061 to 960 g/L for the concentrates and 0.00075 to 250 g/L for the dilutions.

98 **Table 1: Grouping of formulation types and codes used in this study**

Grouping of formulation types	Formulation Code	Number of studies (# of study identifiers)		Number of final observations (mean values)		Description for formulation code
		Dataset ProHuma	Combined ProHuma/ ECPA dataset	Dataset ProHuma	Combined ProHuma/ ECPA dataset	
Organic solvent-based	EC	118	183	316	484	Emulsifiable concentrate
	EW	9	20	25	53	Emulsion, oil in water
	SE	20	45	68	138	Suspo-emulsion
	DC	2	2	5	5	Dispersible concentrate
	OF	1	1	2	2	Oil miscible liquids
	OD	7	22	23	66	Oil-based suspension concentrates
	ES	2	3	4	6	Emulsion for seed treatment
	ME	6	8	20	26	Microemulsion
Water-based /dispersed	SL	21	33	65	96	Soluble concentrate
	SC	127	217	381	606	Suspension concentrate
	FS	46	60	84	116	Flowable concentrate for seed treatment
	CS	13	14	22	25	Capsule suspension
	ZC	4	7	12	18	Mixture of capsule suspension and suspension concentrate
	FL (=SC), SD	1	1	1	1	Flowable
Solids	WP	4	11	10	26	Wettable powder

	WG/WDG	74	108	211	303	Water-dispersible granules
	SG	4	8	9	17	Water-soluble granules
	WS	1	1	2	2	Water dispersible powder for slurry treatment
	GR	1	1	1	1	Granule
Other types	RB	5	8	5	8	Bait, ready for use
	LS	1	1	4	4	Seed treatment
	UL	1	1	1	1	Ultra-low volume
	CB	0	1	0	1	Bait concentrate
	DS	0	1	0	1	Powder for dry seed treatment
	GEL	0	2	0	2	-

CS and ZC formulation type were considered as water-based formulations based on Manual on Development and Use of FAO and WHO Specifications for Pesticides (2016), and ABNT NBR 12679: 2018.

2.2. Data evaluation

The data were evaluated to *in fine* determine default values with the potential relationship between dermal absorption and different parameters: physical form of the formulation, the concentration of active substance, the formulation type, and the molecular weight or the partition coefficient octanol/water (log P_{ow}) of the active substance. Data are presented in various types of graphs / charts including boxplots. The different boxplots show the minimum value, the 25th percentile (lower end of the box), the median (horizontal line within the box), the 75th percentile (upper end of the box) and the maximum value. The 95th percentile of the distributions was consistently superimposed as a red dot/triangle symbol.

The dermal absorption was generally calculated as follows:

Dermal absorption = [Radioactivity present in receptor fluid + receptor chamber wash + whole skin sample] - [radioactivity in the first two tape strips]

2.3. Strength of dataset and exclusion of studies

The dataset is considered homogeneous, robust, and representative of most of all plant protection products since the following criteria from the main regulatory bodies are met:

- *in vitro* dermal absorption studies on human skin;
- performed with agrochemicals products;
- compliant to the OECD 428 with human skin, with the recommended recovery criteria (90-110%);
- conducted under GLP;
- exposure periods of 6 to 10 hours (representative of a working day);
- period of the experiment not exceeding 24 h;
- individual values for tape strips 1 and 2 reported;
- did not have the external addition of surfactant or adjuvants.

Initially, the ProHuma dataset included 488 studies; however, to meet above criteria, 20 studies were excluded from further evaluation. Additionally, studies were excluded based on the following: formulation type was “dry residue” or “tank mixture” or any formulation which wasn’t in its final form for use. Steps involved in the data processing are presented in the Appendix A in Supplementary Material.

2.4. Statistical analysis and software

The data were extracted from study reports, summarised and inserted into a spreadsheet (Microsoft Excel, 2016). This spreadsheet was then read and analysed into the statistical programming language R (RStudio version 1.3.1093 (2020-09-17); R-4.0.3 (October, 2020)). The graphs, medians and percentiles (25th, 75th and 95th) were created and calculated using the RStudio and statistical functions. Graphs and boxplots were created using ggplot2.

Significant differences between groups were determined using ANOVA and Post Hoc Test (Tukey HSD). Shapiro and Levene tests were used in order to show normal distribution and equality of variances. Normal distribution was not shown, and/or variances were heteroscedastic for both datasets. The log transformation provided normally distributed data only for some parameters (e.g. dermal absorption of concentrates but not dilutions). In order to account for potential divergence of the parameters examined a second set of statistical analysis was performed. The Kruskal-Wallis test and multiple comparison after Kruskal-Wallis was

applied in addition to cover non-normal distribution and non-equality of variances, which represents a new contribution of this work in comparison to previous publications. The analysis of variance as well as the Kruskal Wallis test are techniques that aim to verify the association between dermal absorption and factors of interest (*e.g.,* solids and liquids, types of formulations, concentrations, etc.).

The non-parametric methods were chosen because they return better statistical power when the data analyzed is non-normally distributed (parametric data), which is an improvement proposed by this project (Kaur, A., & Kumar, R. (2015)).

Additionally, graphical methods like the Q-Q plot were used. A Q-Q plot (Quantile-Quantile plot) is a graphical tool used to compare two probability distributions by plotting their quantiles against each other. It is also known as a probability plot or normal probability plot. The quantiles are calculated in the sample data and plotted against actual values of quantiles produced by a normal distribution to check if the sample data is normal or not.

3. Results

The following sections will present the findings from the combined ProHuma/ECPA dataset.

3.1 Assessment of dataset distribution

A Q-Q Plot produced by the R-studio platform of the dermal absorption data from the concentrates and dilutions from the combined dataset seems to follow a normal distribution to a greater extend but varies at the borders (**Figure 1**). When using the Shapiro-Wilk normality test a p-value of <0.001 is calculated indicating no normal distribution of dermal absorption data for concentrates and dilutions. When these data are transformed to logarithmic values a normal distribution is visible for the dermal absorption values for the concentrates, but not for dilutions. Similar observations were found in the ProHuma dataset. The equality of variances was proven for comparison of the log-transformed dermal absorption data for the concentrates using the Levene test, but was not proven for the dilutions.

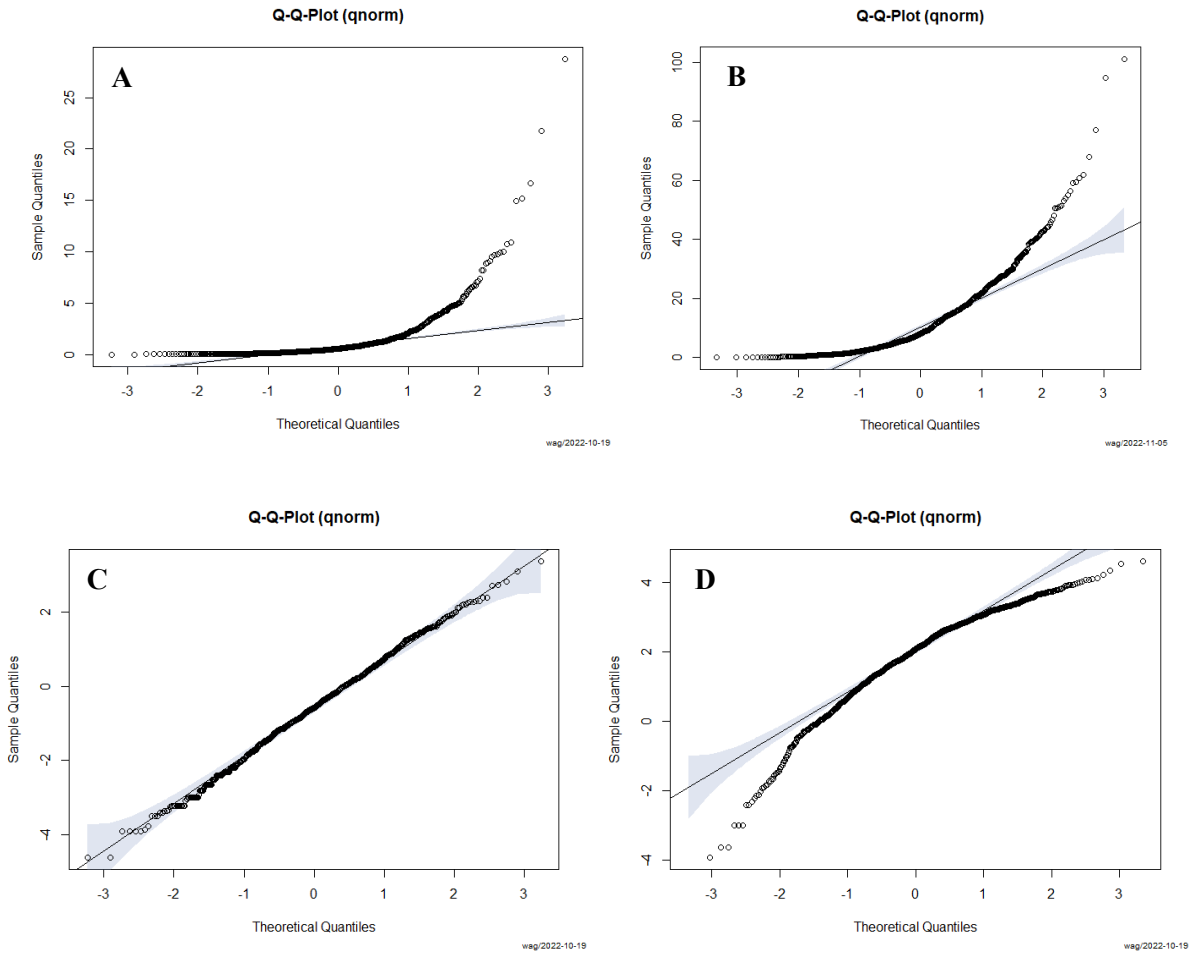


Figure 1: Q-Q-Plot of dermal absorption values (A, B) and Q-Q-Plot of log-transformed (C, D) for concentrates (A, C) and dilutions (B, D) of the combined ProHuma/ECPA dataset (grey: 95th confidence interval)

3.2 Dermal absorption of concentrates and dilutions

Dermal absorption was evaluated for two different definitions for 'concentrates' and 'dilutions': the first definition is based on the fact if concentrated or diluted formulation was tested and the second definition is based on EFSA (SANTE/2018/10591) that considers the content of active substance in the formulation – <5% or 50 g/L was categorized as dilution, whereas a concentration of >5% or 50 g/L was categorized as concentrate.

Considering the first definition and all formulation types, the 95th percentile of the dermal absorption was 4.65% for concentrates and 34.7% for the dilutions for ProHuma dataset and 4.77% for concentrates and 34.1% for dilutions for the combined ProHuma/ECPA dataset (**Figure 2**).

For the second definition, the 95th percentiles were 3.84% for concentrates and 33.0% for dilutions for ProHuma dataset and 4.27% for concentrates and 33.0% for dilutions for the combined dataset.

In this project, if not stated otherwise, the definition for concentrates is the undiluted formulation, no matter its concentration.

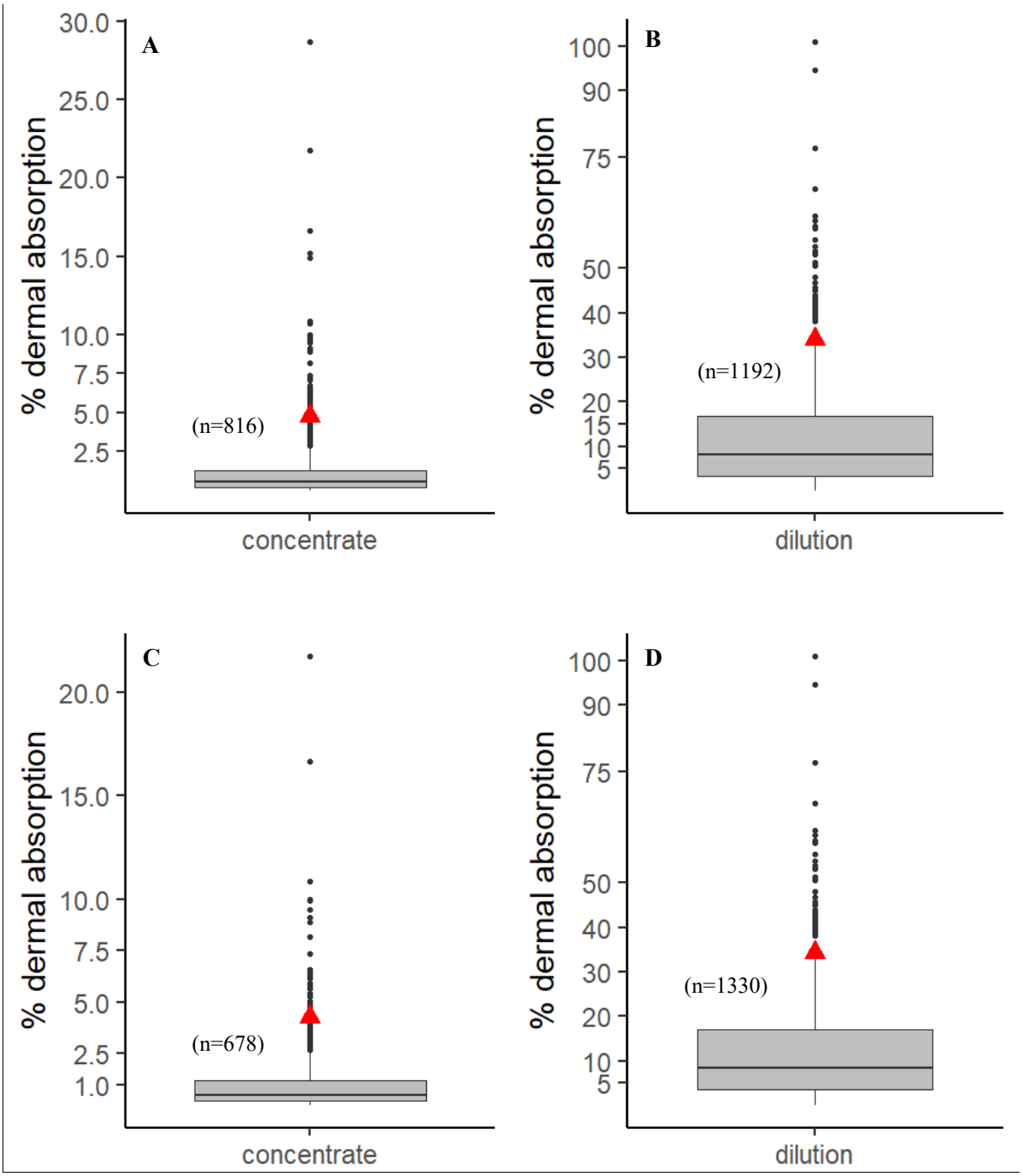


Figure 2: Dermal Absorption (DA) of concentrates (left) and their spray dilutions (right) from combined ProHuma/ECPA dataset. A, B: definition for "concentrate" and

"dilution" is based on the fact that concentrated or diluted formulation was tested; C, D: Definition for "concentrate" and "dilution" was based on the content of active substance.

Two values of dermal absorption from the dilutions from ProHuma dataset were remarkably different from the rest of the data with results close to 100%. These two values, as well as another value of 68% came from one single study with a SC formulation. Such high dermal absorption is not expected and do not seem to represent the whole analyzed set. The study owner has extensively evaluated the report and no technical reason was found to justify the exclusion of this data from the assessment – some possible reasons were raised but all of them were promptly refuted by comparison with other data from the dataset. It was concluded that these results can't be explained by the knowledge available so far and can be attributed to the inherent random behaviour expected when a large number of data, as this one, is handled.

3.3. Effect of physical form (liquid/solid)

The 95th percentile for the ProHuma dataset was 4.72% for liquid concentrates, 1.41% for solid concentrates, 35.8% for liquid dilutions and 25.5% for solid dilutions. For the combined dataset, dermal absorption were 4.85% and 1.72% for liquid and solid concentrates (**Figure 3**), and 34% and 30% for liquid and solid dilutions, respectively (**Figure 4**).

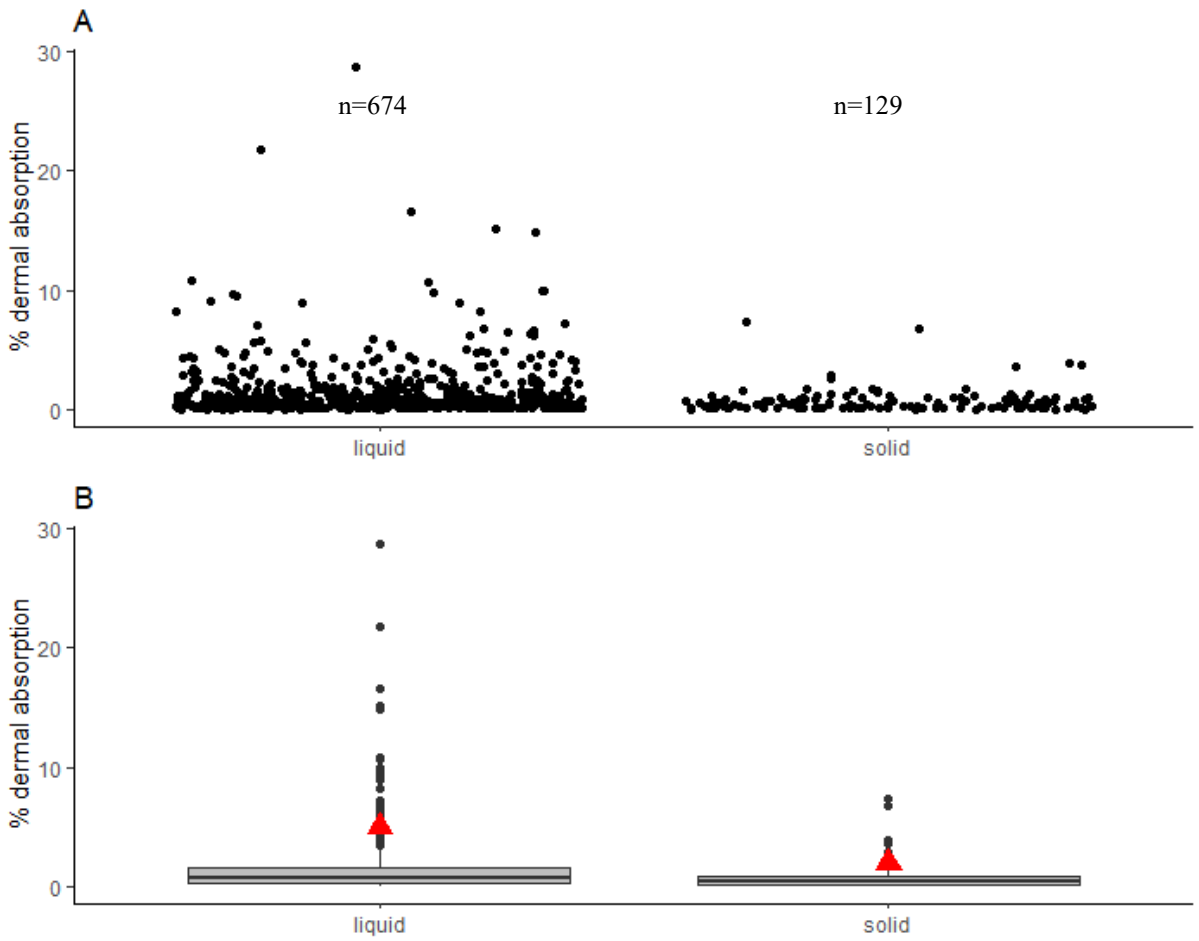


Figure 3: Dermal absorption of concentrates either by scatter (A) or boxplot (B) with superimposed 95th percentiles (red triangle) by physical state from the combined ProHuma/ECPA dataset.

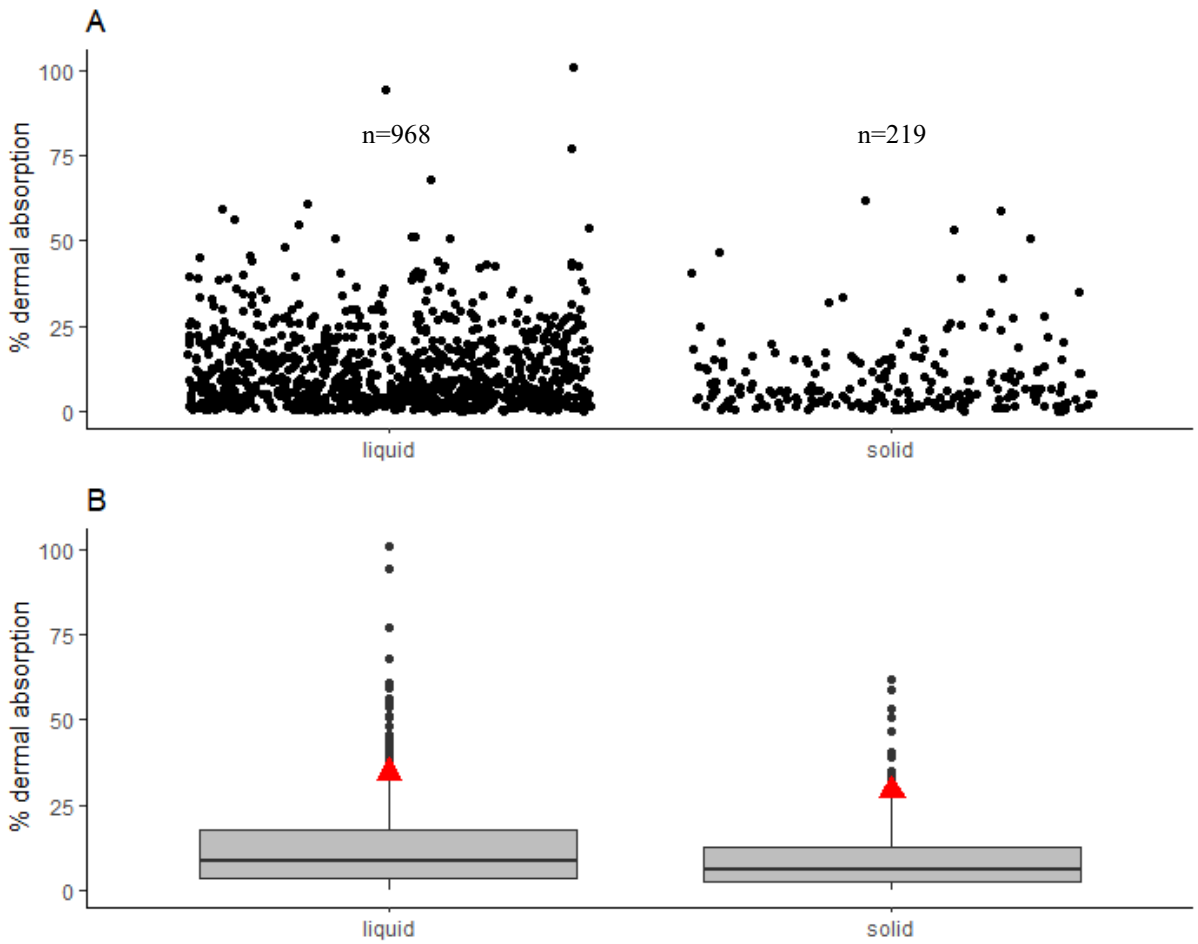


Figure 4: Dermal absorption of dilutions either by scatter (A) or boxplot (B) with superimposed 95th percentiles (red triangle) by physical state from the combined ProHuma/ECPA dataset.

3.4. Effect of active substance concentration

The influence of active substance concentration on dermal absorption was assessed (Appendix B in Supplementary Material). For concentrates and dilutions (undiluted/diluted formulations), the 95th percentile dermal absorption values were 4.77% and 34.1% for the ProHuma/ECPA combined dataset.

When the dermal absorption values are calculated based on the categorisation of concentrates and dilutions according to SANTE/2018/10591, the numbers are slightly different. The dermal absorption values from concentrates (defined as ≥50 g/L active substance) and dilutions (defined as <50 g/L active substance) were 4.27% and 33.0% (95th percentile) for the ProHuma/ECPA combined dataset. In fact, a higher variability of the dermal absorption values

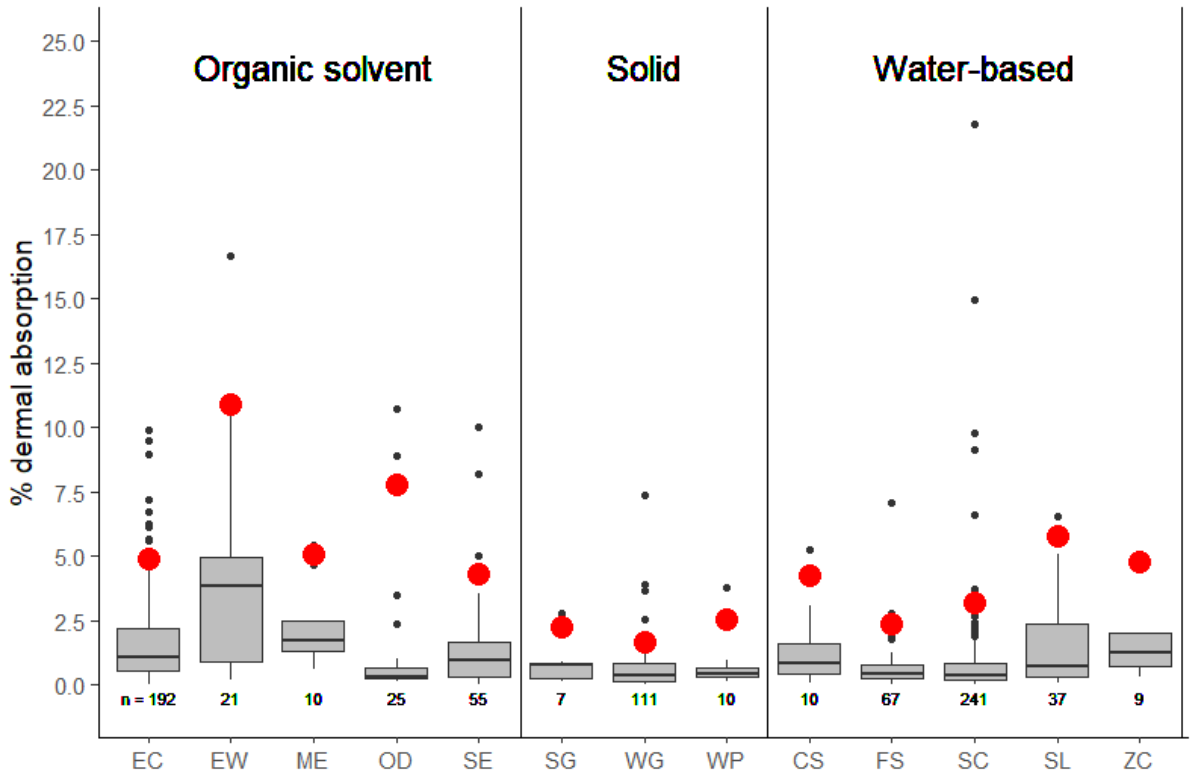
is visible below 5% (50 g/L or g/kg), however, a relevant increase of the values is only observed at concentrations ≤0.5% (5 g/L or g/kg).

3.5. Effect of formulation type

3.5.1. Concentrates

The potential impact that individual formulation type and formulation-type groupings have on dermal absorption from concentrates was assessed (Appendix C in Supplementary Material).

At 95th percentiles, the dermal absorption for organic solvents, solids and water-based formulations were 4.95%, 1.41% and 4.00%, respectively for the ProHuma dataset. For the combined ProHuma/ECPA dataset, 95th percentiles were 5.96, 1.72 and 3.58% for organic solvents, solids and water-based formulations, respectively (**Figure 5**).



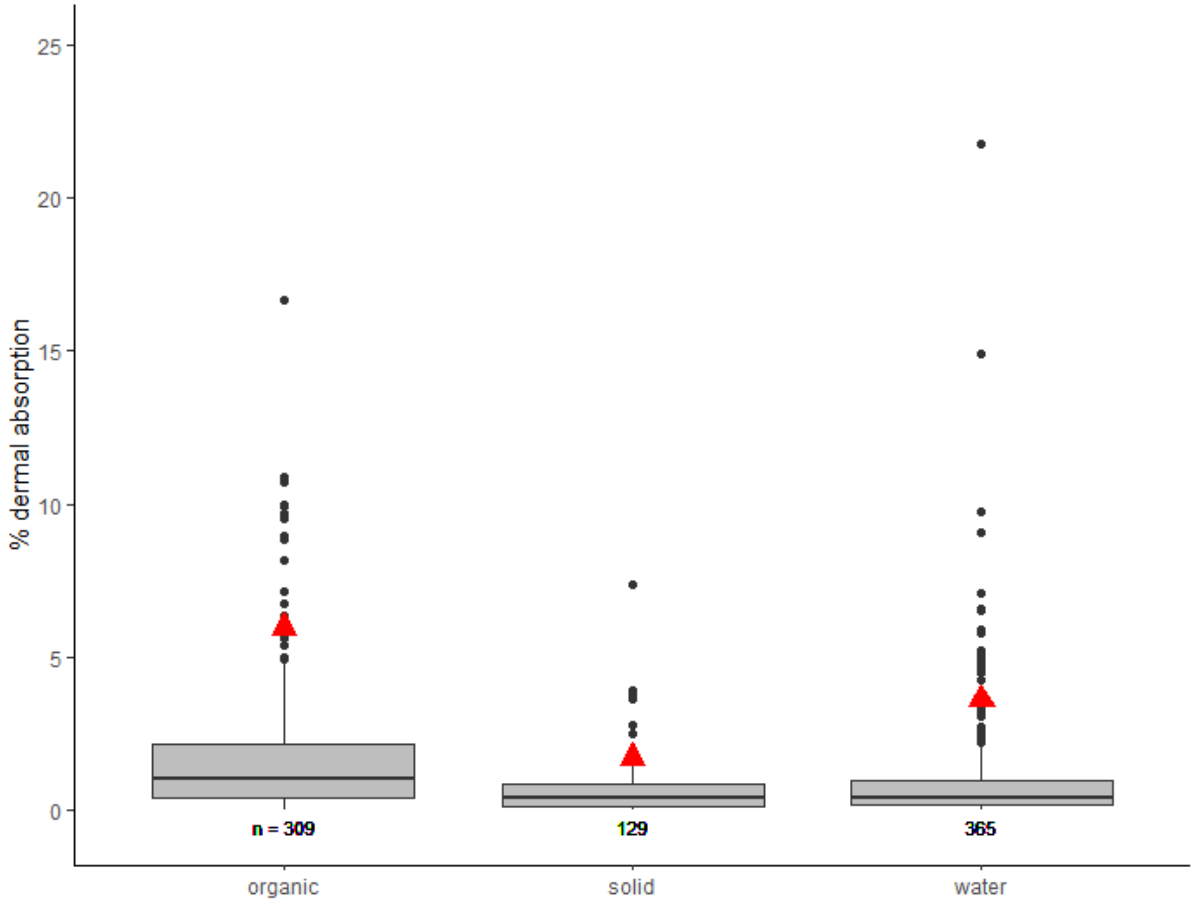


Figure 5: Dermal absorption of concentrates by formulation type (top) and grouped formulations (bottom) with superimposed 95th percentiles (red symbol) – combined ProHuma/ECPA dataset. Only formulation types with n ≥ 7 were included for this evaluation.

Furthermore, the single formulation types within the formulation groups organic, solid and water-based are indicating consistent results. The 95th percentiles are generally comparable and differences are considered due to low number of observations for various formulation types rather than a substantial effect of the formulation type *per se*.

At 95th percentiles, EC, ME, OD, and SE organic formulations had similar dermal absorption values of 4.87, 5.08, 7.79 and 4.29%. EW formulations showed relatively higher dermal absorption (10.9%) but 'n' was rather small (n=21). EW and OD showed higher variations mainly due to some extreme values and thus higher overall 95th percentiles in the ProHuma dataset and combined ProHuma/ECPA dataset. A statistical difference ($p<0.001$) was observed between EC, SE, OD compared to EW using ANOVA and Tukey-HSD. Using Kruskal-Wallis

257 and then multiple comparisons, no differences were observed between the various formulation
258 types (at p=0.05) except for EW and OD when compared to EC, ME and SE.

259 At 95th percentiles, SG, WG, and WP solid formulations had similar dermal absorption values
260 of 2.2, 1.7, and 2.5%. Consistent with the previous datasets (Aggarwal *et al*., 2015), solid
261 formulations had relatively low dermal absorption values. No statistical difference was
262 observed between solid formulation types (p>0.05) using ANOVA and Kruskal-Wallis tests.

263 At 95th percentiles, FS and SC water-based formulations had similar dermal absorption values
264 of 2.38 and 3.18%. For CS, SL, and ZC slightly higher dermal absorption values of 4.26, 5.80,
265 and 4.78% was observed. Consistent with the previous datasets (Aggarwal *et al*., 2015), water-
266 based formulations in general had relatively low dermal absorption values when compared to
267 organic-solvent based. No statistical difference was observed between water-based formulation
268 types (p>0.05) using ANOVA and Kruskal-Wallis tests.

269 **3.5.2. Dilutions**

270 As presented for the concentrates, a formulation type can significantly influence dermal
271 absorption. Therefore, the potential impact that individual formulation type (**Figure 6**) and
272 formulation-type groupings have on dermal absorption from spray dilutions was assessed
273 (Appendix C in Supplementary Material).

274 The 95th percentiles for organic solvents, solids and water-based formulations were 35.5, 29.0
275 and 30.2%, respectively.

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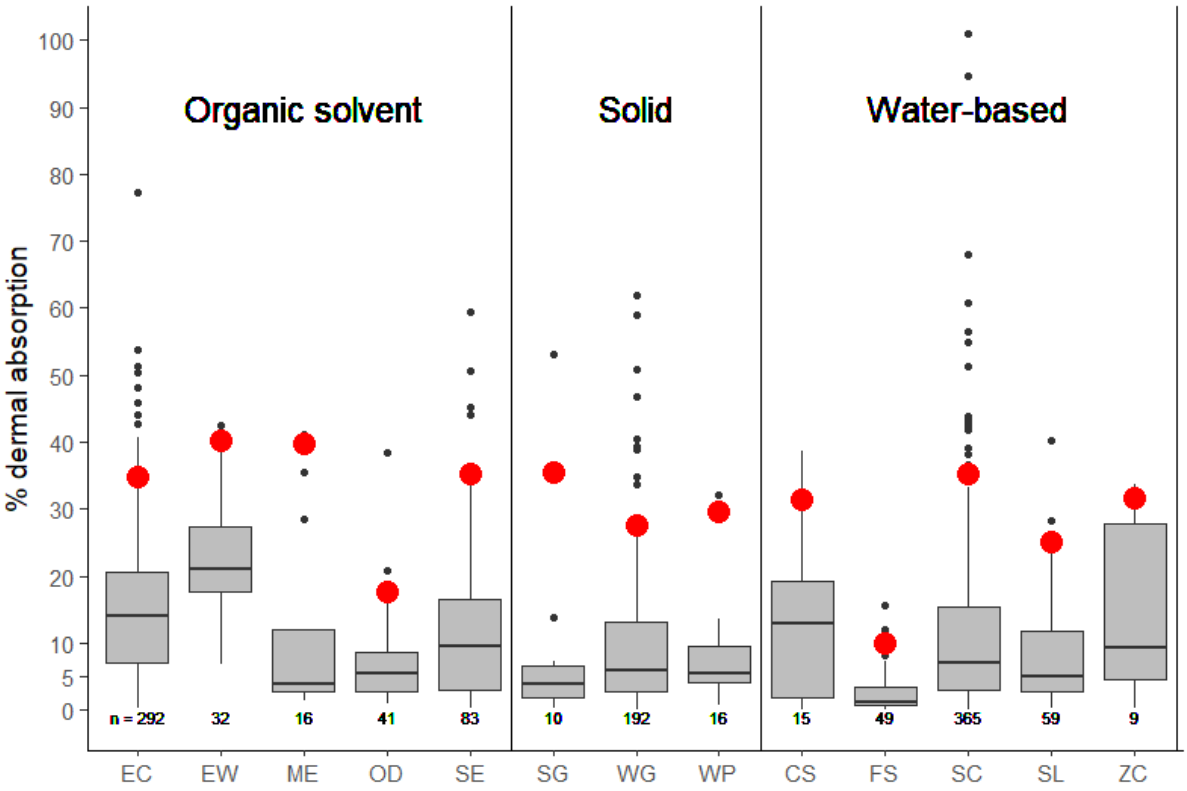
Organic solvent
Solid
Water-based
% dermal absorption
100
90
80
70
60
50
40
30
20
10
5
0
n = 292
32
16
41
83
10
192
16
15
49
365
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9
EC
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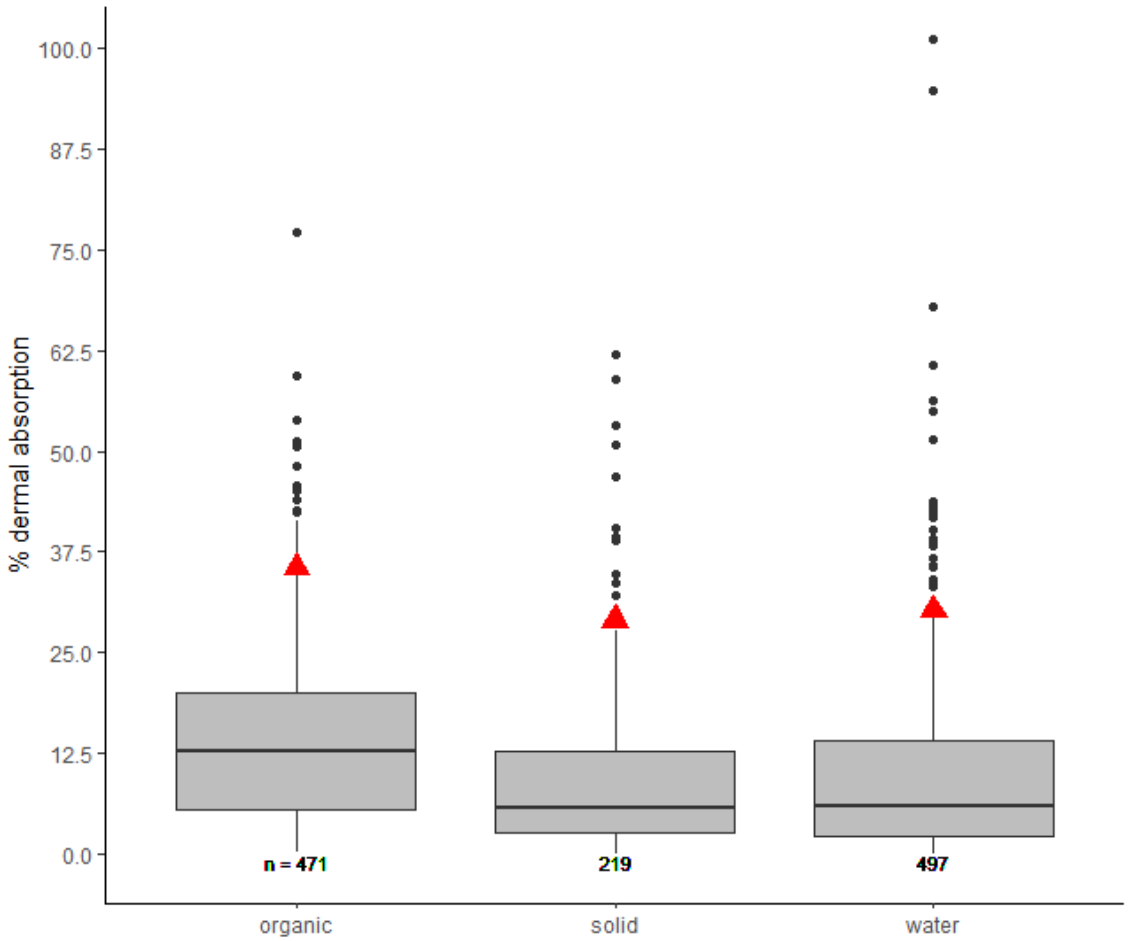

% dermal absorption
100.0
87.5
75.0
62.5
50.0
37.5
25.0
12.5
0.0
n = 471
219
497
organic
solid
water

278

Figure 6: Dermal absorption of dilutions by formulation type (top) and and grouped formulation (bottom) from the combined ProHuma/ECPA dataset with superimposed 95th percentiles (red dot). Only formulation types with n ≥9 were included for this evaluation.

At 95th percentiles, organic solvent-based formulations EC, EW, ME and SE had similar dermal absorption values of 34.8, 40.3, 39.7, and 35.2% whereas for OD a smaller value of 17.6% was observed. A statistical difference ($p<0.001$) was observed between SE and OD compared to EW (but not EC ($p=0.052$)) using ANOVA. The Kruskal-Wallis test showed a significant difference (at $p=0.05$) for SE, OD as well as ME. A statistical difference was observed between OD ($p=0.0064$, ANOVA) compared to EC. The statistical significance for this difference between EC and OD was supported by Kruskal-Wallis test.

SG, WG, and WP solid formulations had similar dermal absorption values of 37.4%, 27.8 and 29.7%. No statistical difference was observed between solid formulation types ($p>0.05$) using ANOVA and Kruskal-Wallis tests.

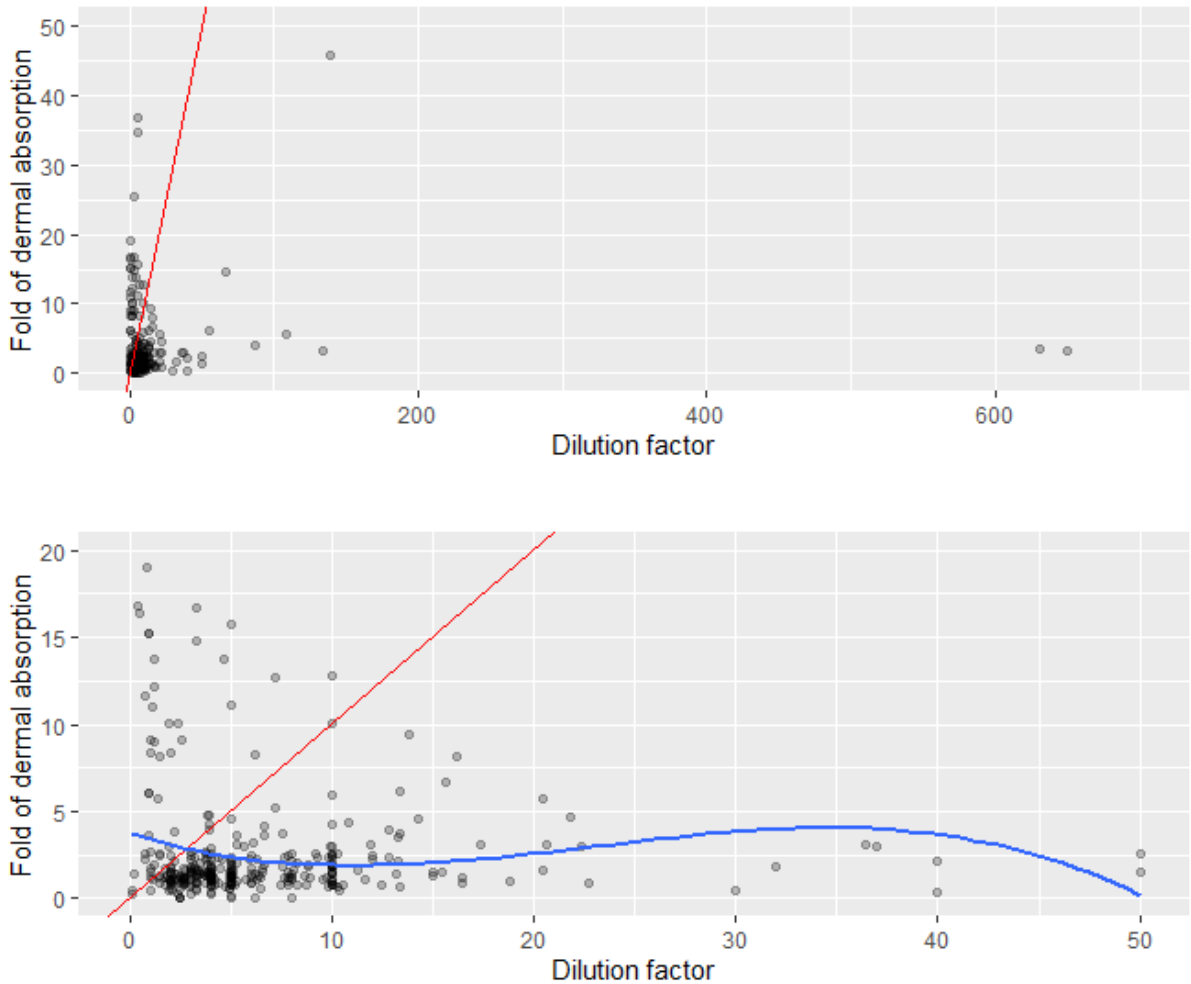
CS, SC, SL, and ZC water-based formulations had similar dermal absorption values of 31.5, 35.1, 25.1, and 31.6%. For FS, a dermal absorption value of 9.9% was observed. Based on statistical analysis the FS was significantly different ($p<0.001$) from SC but not from CS, SL or ZC using ANOVA. The Kruskal-Wallis test revealed a difference between FS and the other formulation types including CS, SC, SL, ZC, respectively. The difference for FS can be attributed to the fact that this type of formulation is intended for seed treatment and thus usually only marginally diluted.

3.6. Relationship between molecular weight or log P_{ow} and dermal absorption

The current evaluation for the combined database also evaluated the potential relationship between dermal absorption and molecular weight or log P_{ow} (Appendix D in Supplementary Material). Various regression models were applied and no model showed any reliable relationship and thus no dermal absorption default value could be proposed based on these parameters. This finding is aligned to what was previously concluded by EFSA (2017) and Aggarwal *et al.,* 2015.

3.7 Effect of factor of dilution on dermal absorption

Pro rata correction applied by EFSA (2017) for the extrapolation from a measured dilution to a higher non-measured dilution assumes a linear response. In the combined dataset, 356 ratios of lower dilutions/higher dilutions are presented ("Dilution factor" *vs* "Fold of DA")[7] (**Figure 7**). Other relationships such as fold of dermal absorption versus dilution were assessed and in all evaluations the dataset suggests no linear relationship, a similar conclusion was reported by Aggarwal *et al*. 2015. The increase of dermal absorption with dilution is considerably smaller than predicted by the pro rata approach – in 87% of the cases, the increase is less than 5-fold of dermal absorption (**Figure 7**, bottom). The stepwise procedure for extrapolation of dermal absorption data proposed by Aggarwal *et al*. (2015) is meaningful and can be found in Appendix E of the Supplementary Material.



[7] Dilution factor = concentration [g/L] of lower dilutions / concentration [g/L] of higher dilution; Fold of DA = DA of dilution / DA of concentrate

Figure 7: Dilution factor (x-axis) plotted against the fold of dermal absorption (y-axis) (only dilutions were considered) from combined ProHuma/ECPA dataset. All ratios (n=356) are presented in the plot. (Blue line: polynomial regression from current dataset; Red line: showing regression according EFSA pro rata approach). Intensity color of points indicates higher numbers of observations.

4. Discussions

4.1 Dermal absorption of concentrates and dilutions

In the EFSA guidance, default values for dermal absorption are provided for concentrates (defined as ≥50 g/L active substance) and dilutions (defined as <50 g/L active substance) for two main formulation categories (organic solvent-based or other, and water-based/dispersed or solid).

No relevant differences in dermal absorption were obtained when different definitions of concentrates and dilutions were taken into account. The data indicate that two different defaults for concentrates based on active substance content are not justified.

4.2 Effect of physical form (liquid/solid)

Taking into account the ProHuma dataset and the ProHuma/ECPA combined dataset, two different default values can be proposed for concentrates: 5% for liquids and 2% for solids.

For spray dilutions no difference is generally expected if the dilution is based on a solid or liquid concentrate. However, a difference between these two types was observed. No final conclusion can be made why the dermal absorption of solid dilutions is lower than for liquid dilutions. Probably that in dilutions from solids not all parts are completely solubilized, thus having an effect on the absorption. Therefore, for dilutions, two default dermal absorption values are proposed: 34% for liquids and 30% for solids. For future work, it is recommended that this difference be assessed via methods that describe the actual probability distribution for each category considered, which may be different given the substantial difference between the percentiles considered.

4.3 Effect of active substance concentration

As aforementioned, the definition adopted in this project for ‘concentrates’ is the undiluted formulation. Even though, the effect of active ingredient loading on dermal absorption values was assessed.

It has been shown that a meaningful increased dermal absorption can be expected at active substance concentrations <5 g/L, which cannot be excluded completely for concentrates as well. As most concentrates (undiluted product per definition) contain more than 5 g/L of active substance, the use of one default value for concentrates is considered meaningful. Only 11 out of 821 tested concentrates contained less than 5 g/L of active substance. Otherwise, the definition for concentrates and dilutions could be based on the content of more or less than 5 g/L instead of 50 g/L as adopted by EFSA. Based on this, the definition of concentrates as the undiluted formulation can be considered as an appropriate approach.

4.4 Effect of formulation type

For the combined dataset, ANOVA found a pronounced statistically significant grouping effect on dermal absorption ($p \leq 0.001$). Tukey-HSD post hoc analysis (percentiles) revealed significant group differences ($p < 0.001$) between organic solvent-based and solid as well as between organic solvent-based and water-based; additionally, water-based formulations and solids were not significantly different. Using multiple comparison test after Kruskal-Wallis the results were comparable indicating a statistically significant difference between organic solvent-based and solid as well as water-based formulations at $p=0.05$ but indicated no difference between water-based formulations and solids.

As described in Aggarwal *et al*. (2015), the higher dermal absorption observed for organic solvents concentrates may be due to the fact that most of the active substances are solubilised in the organic solvent and are immediately available for dermal penetration.

4.5 Opportunities for read-across

The statistical evaluation performed found a pronounced statistically significant effect of grouping on dermal absorption and revealed significant group differences between organic-solvent based and both water-based and solid formulations; water-based formulations and solids were not significantly different. Dermal absorption data generated with a solvent-based formulation could be used as conservative surrogate for primarily water-based and solid formulations. Similarly, dermal absorption data generated with water-based formulations could be used as a surrogate for solid formulations.

5. Conclusions

In this project, 488 studies (ProHuma dataset) were gathered and combined with the dataset from the ECPA, generating a dataset (ProHuma/ECPA combined dataset) with 759 GLP compliant *in vitro* dermal absorption studies using human skin that followed OECD 428 guideline. From these studies a huge and homogeneous database was built with 2008 observations in total, increasing significantly the number of observations per formulation type. After the evaluation of this database, it can be concluded that it is sufficiently robust to derive reliable conclusions on dermal absorption, such as default values and read-across possibilities.

Comparisons were done between ECPA database, ProHuma database and the combined ProHuma/ECPA database. These assessments are the basis for the conclusions that were drawn and presented in this project.

Following the discussion of the effect that different parameters have on dermal absorption, and according to the rationale presented by Aggarwal *et al.* (2015), a proposal for default values based on the 95th percentile is provided for solid/liquid concentrates as well as for solid/liquid dilutions (Table 2), applicable to all formulation types.

Table 2: Suggested default values for dermal absorption of concentrates and dilutions by physical form (95th percentile) based on ProHuma/ECPA dataset

	Concentrate	**Dilution**
Solid formulation	2%	30%
Liquid formulation	5%	34%

The proposal based on the combined ProHuma/ECPA dataset does not differ significantly from the 95th percentiles obtained from the ProHuma dataset and from the proposed defaults from Aggarwal *et al.*, 2015 (Appendix F in Supplementary Material).

The read-across potential is proposed based on the statistical evaluation from groupings and also by considering the statistical differences of dermal absorption within each group:

Read-across potential	Organic-solvent based DA values can be used as conservative surrogates for solid and water-based formulations.
	Organic-solvent and water-based DA values can be used as conservative surrogates for solid formulations.

Read-across within the same formulation group can be done on a case-by-case basis, always considering the 95th percentile most-conservative value as a surrogate.

Credit authorship contribution statement

Danilo Sarti: statistical analysis, writing – reviewing; **Jörg Wagner:** statistical analysis, writing – original draft, preparation, reviewing & editing; **Fabiana Palma:** project administration, writing – reviewing & editing; **Marcela Giachini:** writing – reviewing & editing; **Daniele Lautenschalaeger**: writing – reviewing & editing; **Janaina Pires**: writing – reviewing & editing; **Marina Sales**: writing – reviewing & editing; **Patricia Faria**: writing – reviewing & editing; **Vanessa Lupianhez**: writing – reviewing & editing; **Heloisa Kalvan**: writing – reviewing & editing; **Marine Le Bras:** writing – original draft, preparation, reviewing & editing; **Lisa Bertomeu:** writing – original draft, preparation, reviewing & editing.

Declaration of competing interest

The authors declare the following financial/personal relationships which may be considered as potential competing interest: Instituto ProHuma de Estudos Cientificos is a consortium formed by agrochemical companies. Marcela Giachini, Daniele Lautenschalaeger, Janaina Pires, Marina Sales, Patricia Faria, and Vanessa Lupianhez are employees of agrochemical companies.

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References

ABNT NBR 12679: 2018. Agrotoxicos e afins – Produtos tecnicos, concentrados tecnicos e formulacoes – Terminologia.

ANVISA, 2019. Resolução da Diretoria Colegiada – RDC N° 294, de 31 de julho de 2019. *Agência Nac. Vigilância Sanitária*.

Aggarwal, M., Battalora, M., Fisher, P., Hüser, A., Parr-Dobrzanski, R., Soufi, M., Mostert, V., Strupp, C., Whalley, P., Wiemann, C., Billington, R., 2014. Assessment of in vitro human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. Regul. Toxicol. Pharmacol. 68(3), 412-423

Aggarwal, M., Fisher, P., Hüser, A., Kluxen F.M., Parr-Dobrzanski, R., Soufi, M., Strupp, C., Wiemann, C., Billington, R., 2015. Assessment of an extended dataset of *in vitro* human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. Regul. Toxicol. Pharmacol. 72(2015), 58-70

EFSA, 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873.

FAO/WHO, 2016. Manual on development and use of FAO and WHO specifications for pesticides. Pesticide Specifications. 1st ed. 3rd rev.

Kaur, A., & Kumar, R., 2015. Comparative analysis of parametric and non-parametric tests. *Journal of computer and mathematical sciences*, 6(6), 336-342.

OECD, 2004a. Guideline for the Testing of Chemicals Guideline 427: Skin absorption: *in vivo* method (adopted: 13 April 2004).

OECD, 2004b. Guideline for the Testing of Chemicals Guideline 428: Skin absorption: *in vitro* method (adopted: 13 April 2004).

OECD, 2019. Guidance Notes on Dermal Absorption. Series on Testing and Assessment no. 156. Guidance notes on dermal absorption. Draft Second Edition.

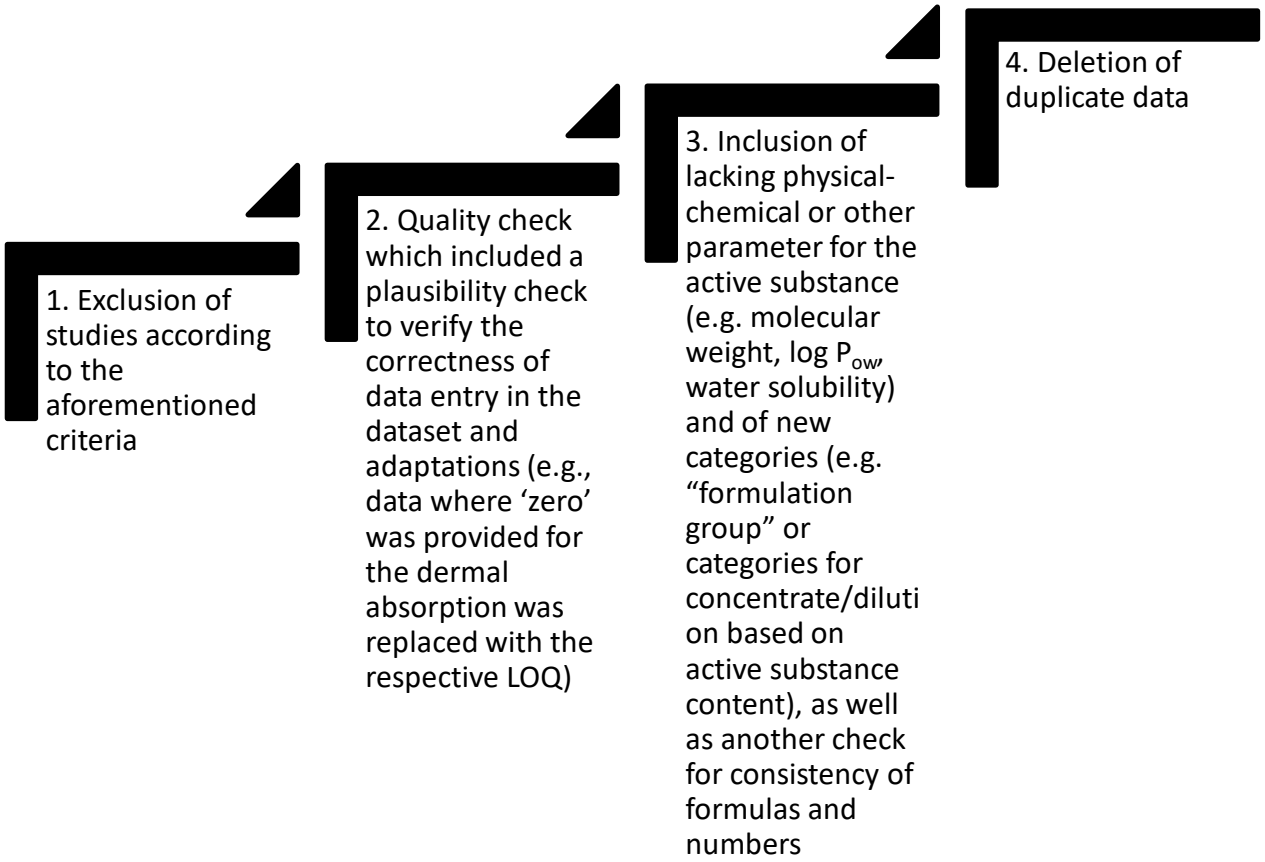
SANTE/2018/10591 (24 October 2018, rev.1). Guidance on dermal absorption. https://food.ec.europa.eu/system/files/2018-11/pesticides_ppp_app-proc_guide_tox_dermal-absorp-2018-paff.pdf

WHO, 2006. Environmental Health Criteria 235: Dermal Absorption.

Supplementary Material

The following is the Supplementary data to this project.

Appendix A. Steps involved in the data processing



Appendix B. Effect of active substance concentration

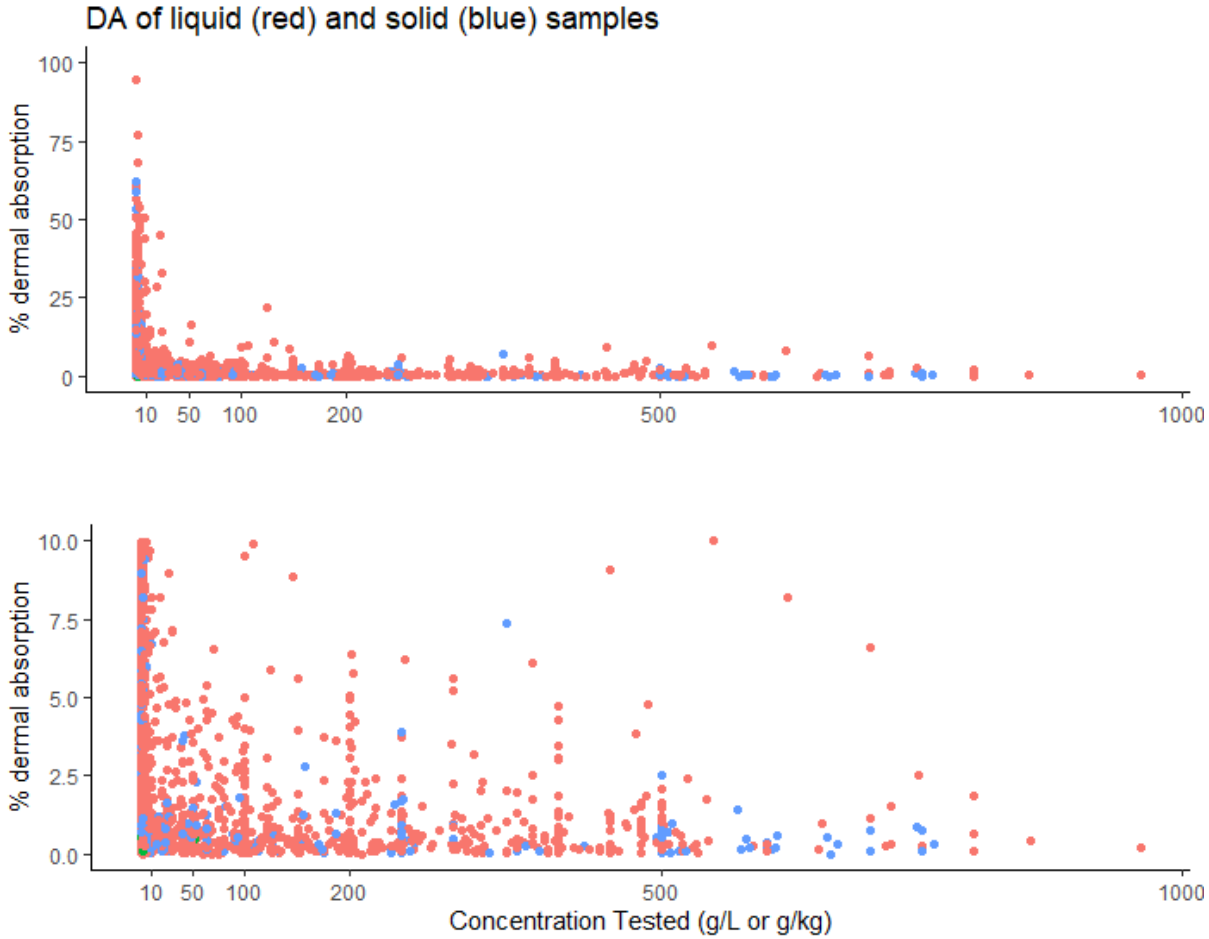


Figure 1: Dermal absorption scatter plots from samples throughout the full concentration range from combined ProHuma/ECPA dataset. In the upper part of the graph all values are shown (dermal absorption 0-100%), whereas in the graph at the bottom only a part of the values is shown (dermal absorption 0-10%).

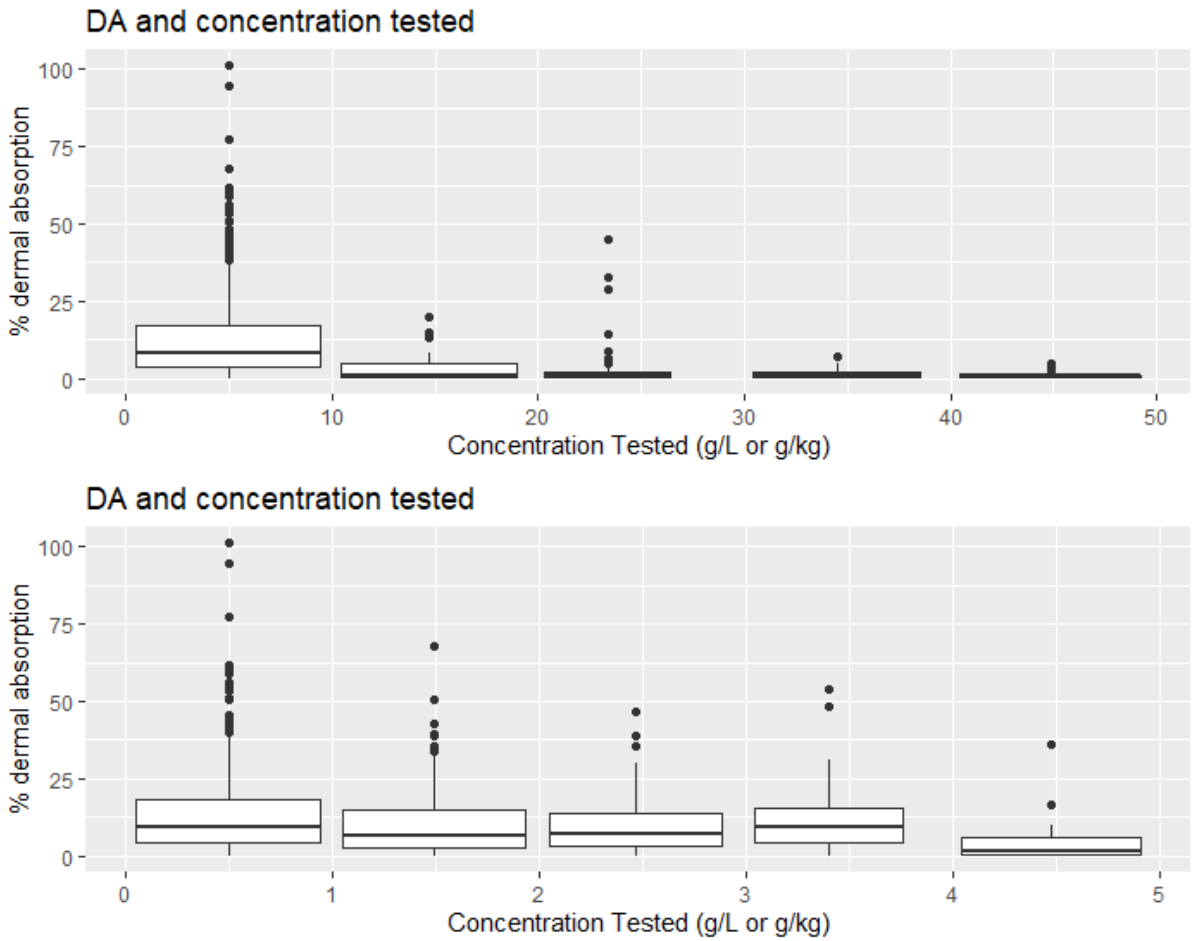


Figure 2: Dermal absorption boxplots of samples with concentrations between 0 and 50 g/L (upper graph) and between 0 and 5 g/L (each divided into 5 sub-ranges with a similar number of data points) from combined ProHuma/ECPA dataset.

Appendix C. Effect of formulation type

Table 1: Overview of dermal absorption's percentiles (%) and number of observations by grouped formulation types for concentrates and dilutions (ProHuma/ECPA dataset)

	Concentrates				Dilutions			
Formulation type	Percentiles (% of dermal absorption)#			Number of observations	Percentiles (% of dermal absorption)#			Number of observations
	95th	75th	50th	N	95th	75th	50th	N
Organic solvent								
DC	-	-	-	2	14.4	13.2	11.7	3
EC	4.87	2.20	1.04	192	34.8	20.5	14.0	292
EW	10.9	4.96	3.86	21	40.3	27.5	21.0	32
ES	0.83	0.81	0.79	3	3.57	2.92	2.10	3
ME	5.08	2.46	1.71	10	39.7	12.1	3.94	16
OD	7.79	0.67	0.29	25	17.8	9.01	5.25	41
OF	-	-	-	1	-	-	-	1
SE	4.29	1.65	0.97	55	35.2	16.6	9.47	83
Solid								
SG	2.23	0.85	0.77	7	37.4	7.21	4.28	10
WG	1.68	0.85	0.38	111	27.8	13.2	6.08	192
WP	2.51	0.63	0.44	10	29.7	9.50	5.42	16
WS	-	-	-	1	-	-	-	1
Water-based/dispersed								
FS	2.38	0.79	0.45	67	9.92	3.44	1.20	49
SC	3.18	0.82	0.39	241	35.1	15.1	6.96	365
SD	-	-	-	1	-	-	-	0
SL	5.80	2.37	0.74	37	25.1	11.8	4.88	59
CS	4.26	1.63	0.81	10	31.5	19.3	13.0	15
ZC	4.78	1.99	1.24	9	31.6	27.9	9.28	9
Other Types								
CB	-	-	-	1	-	-	-	0
DS	-	-	-	1	-	-	-	0
GEL	-	-	-	1	-	-	-	1
LS	-	-	-	2	-	-	-	2
RB	5.21	1.12	0.50	7	-	-	-	1

UL	-	-	-	1	-	-	-	0
GR	-	-	-	0	-	-	-	1

#: calculations were performed if n ≥ 3

Appendix D. Relationship between molecular weight or log P_{ow} and dermal absorption

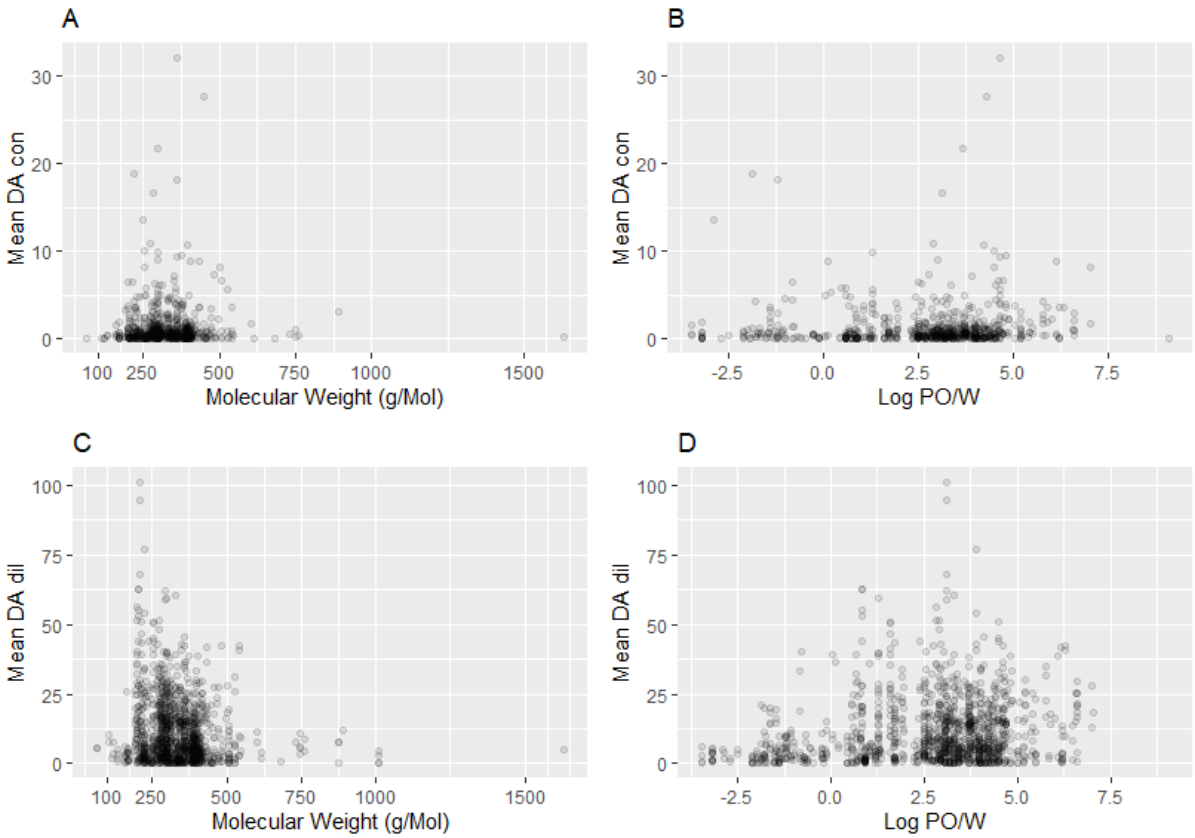


Figure 3: Dermal absorption of concentrates (A, B) and their spray dilutions (C, D) by molecular weight (A, C) or log P_{ow} (B, D) from combined ProHuma/ECPA dataset. Intensity color of points indicates higher numbers of observations.

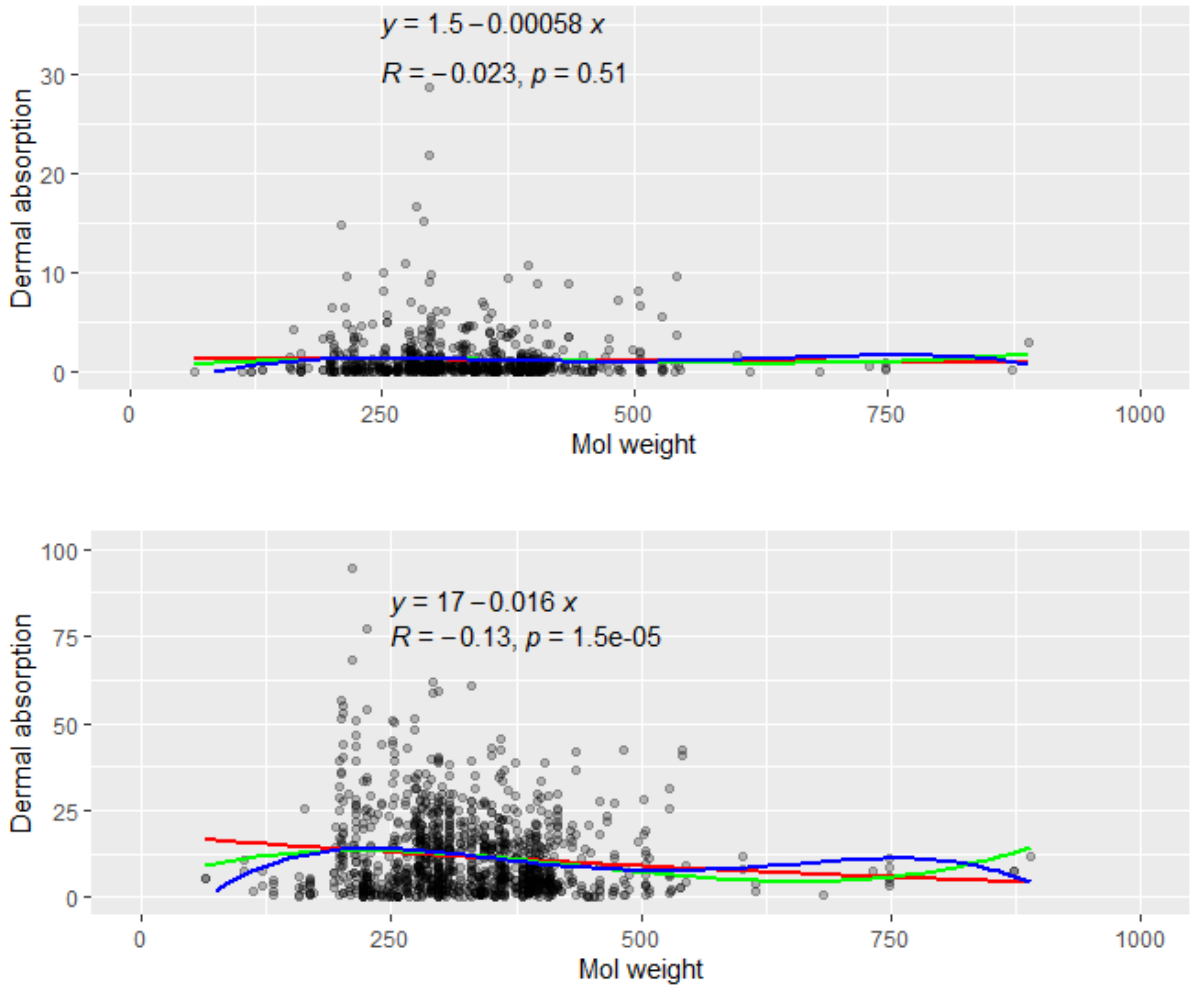


Figure 4: Dermal absorption of concentrates and their spray dilutions (up, below) by molecular weight from combined ProHuma/ECPA dataset. Coloured lines show polynomial regressions (red: poly degree 2, green: poly degree 3, blue: poly degree 4).

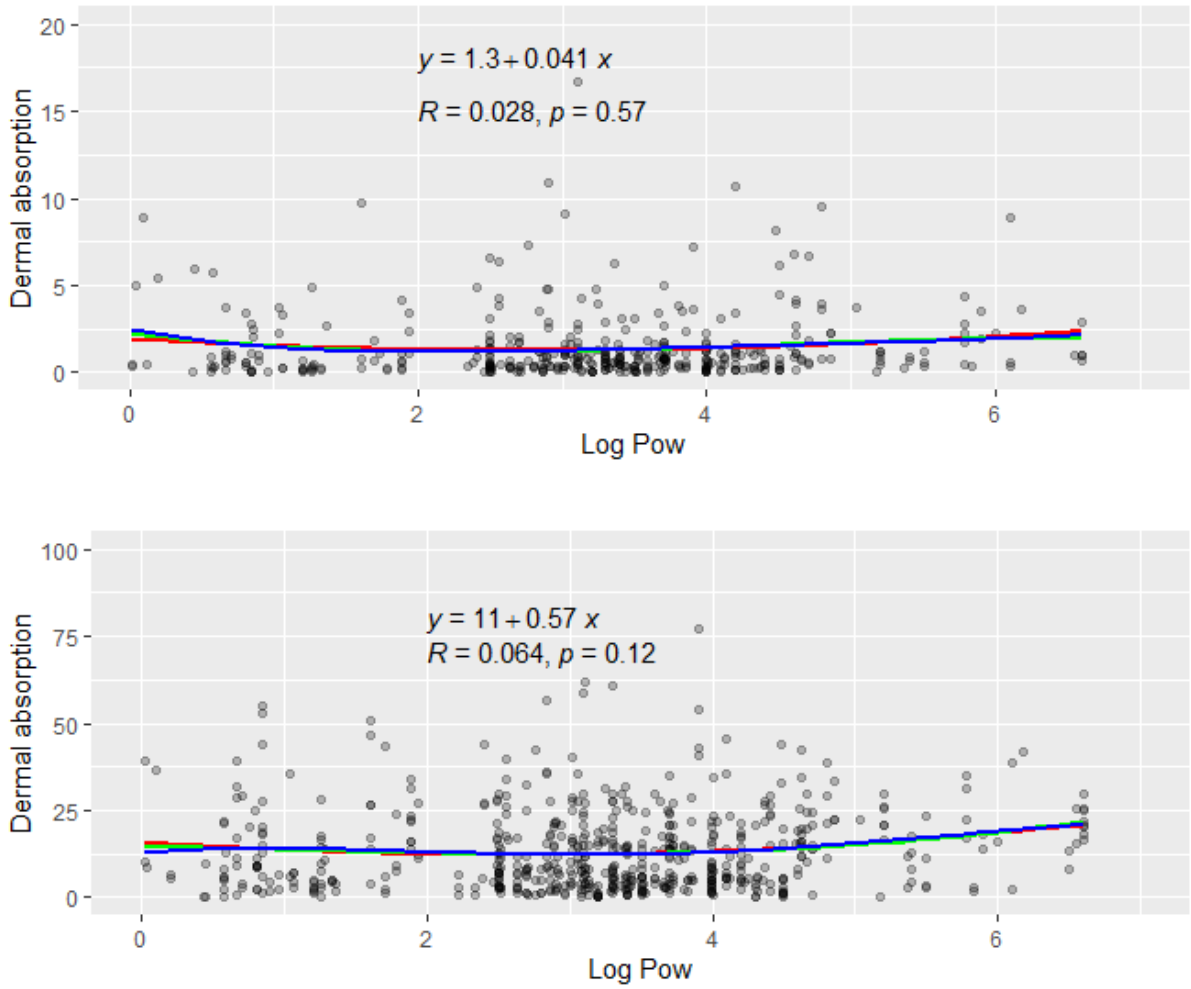
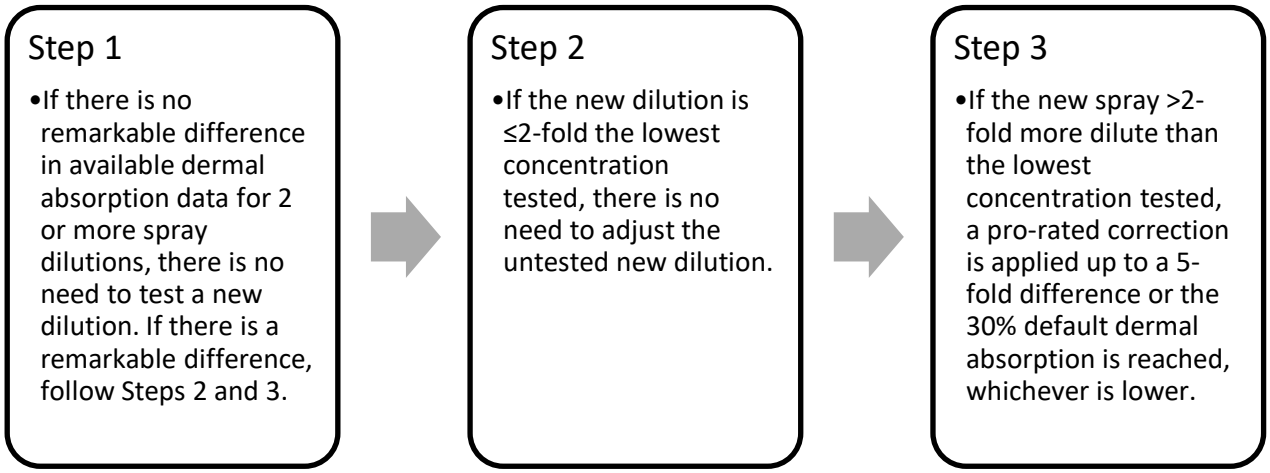


Figure 5: Dermal absorption of concentrates and their spray dilutions (up, below) by Log Pow from combined ProHuma/ECPA dataset. Coloured lines show polynomial regressions (red: poly degree 2, green: poly degree 3, blue: poly degree 4).

Appendix E. Procedure for extrapolation of dermal absorption data (reproduced from Aggarwal *et al*., 2015)



Appendix F. Summary of proposed dermal absorption defaults

Table 2: Comparison of default values for dermal absorption of concentrates and dilutions by physical form (95th percentile)

	ProHuma/ECPA dataset		ProHuma dataset		Aggarwal *et al.* (2015)	
	Concentrate	**Dilution**	**Concentrate**	**Dilution**	**Concentrate**	**Dilution**
Solid formulation	2%	30%	2%	26%	2%	30%
Liquid formulation	5%	34%	5%	36%	6%	